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EVALUATION AND COMPARISON OF CHLORHEXIDINE GLUCONATE BATHING SOLUTION AND ANTISEPTIC WIPES ANTIMICROBIAL EFFECTS AGAINST BACTERIA CAUSING NOSOCOMIAL INFECTIONS

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Abstract: Nosocomial infections among critical patients in intensive care units are associated with significant morbidity and mortality globally, including in Malaysia. Both Chlorhexidine gluconate (CHG) bathing solution and antiseptic wipes were used in preoperative preparation. A controversy was reported on the inhibitory effects of antiseptic wipes and CHG bathing solution on the bacteria causing nosocomial infections. This study was conducted to evaluate the antimicrobial effects of the antiseptic wipes and CHG bathing solution on nosocomial bacteria (methicillin-resistant *S. aureus* (MRSA), *Acinetobacter baumannii, Escherichia coli, Klebsiella* spp. and *Pseudomonas aeruginosa*). The antibacterial effectiveness of antiseptic wipes impregnated with 2% CHG and bathing solution 4% CHG were assessed using agar well diffusion method. Microtiter plate assay was used to estimate the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). The CHG from antiseptic wipes was aseptically extracted by squeezing method. The CHG bathing solution revealed excellent inhibitory effects against all study bacteria with inhibition zones [15 mm (*A. Baumannii*), 17 mm (*Klebsiella* spp.), 20 mm (*Pseudomonas aeruginosa*), 23 mm (*Escherichia coli*) and 25 mm (MRSA). In contrast, antiseptic wipes were effective against *E. coli* only with 15 mm inhibition zone. The MIC of CHG bathing solution was 0.03% for all study bacteria except for *P. aeruginosa,* which was 0.06%; however, the MIC of the antiseptic wipes against MRSA and *E. coli* were 0.13% and 0.5%, respectively. The MBC of CHG bathing solution against MRSA, *A. baumannii* and *E. coli* were 4%, 0.25% and 0.5% respectively. The MBC of antiseptic wipes couldn't be determined since all study bacteria showed uncountable colonies even with the highest concentrations. In conclusion, CHG bathing solution showed a stronger antibacterial effect than antiseptic wipes against nosocomial bacteria. Using CHG bathing solution will significantly reduce the risks of acquiring multidrug resistant organisms and developing nosocomial infections.

Keywords: Antimicrobial, antiseptics, bathing solution, nosocomial, Chlorhexidine gluconate.

Introduction

Chlorhexidine gluconate (CHG) is an antiseptic that can be used on skin and environmental surfaces, and has displayed broad-spectrum activity against several organisms, including *S. aureus*, *Enterococcus* species and multi- drug resistant (MDR) bacteria (Ekizoglu *et al.*, 2016).

CHG alters the surface charge of the bacterial cell by reversing the charge, resulting in damage to the cytoplasmic membrane and leakage of cytoplasmic contents with the loss of low molecular weight molecules (McDonnell & Russell, 2001). The rate of membrane disruption and cell leakage increases with CHG concentration up to a maximum of 100 to 500 m/L, and then fall back and no more leakage occurs (McDonnell & Russell, 2001).

Nosocomial bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*, have become endemic in many health care centers globally including Malaysia (Al-Talib *et al.*, 2019; Sikora & Zahra, 2021). Skin microflora are microorganisms that are resident on our skin. Normal flora in the skin is considered the largest barrier against potentially pathogenic organisms (Abdallah *et al.*, 2017). The FDA-approved CHG as a skin antiseptic is available in a 4% solution that is rinsed off after bathing, and 2% CHG impregnated in wipes. The aim of using CHG in patients subjected to surgery or in intensive care unit (ICU) is to reduce the microbial load on the skin surface to lowest value earlier to surgical intervention, in order to reduce the risk of wound contamination. Furthermore, The Centers for Disease Control and Prevention strongly recommended using antiseptic agent at least the night before the operative day to eliminate harmful bacteria. Daily bathing with CHG has been found to be effective in preventing infections in ICU patients (Climo *et al.*, 2009). Previous studies showed the effectiveness of CHG in bathing solution and wipes in the reduction of hospital-acquired infections (Graling & Vasaly, 2013). However, the in-vitro effectiveness of CHG used in bathing solution, and from wipes against bacteria causing nosocomial infections have not been compared. The aim of this study is to determine the effectiveness of CHG antiseptic wipes and to compare it with CHG bathing solution against bacteria causing nosocomial infections.

Materials and Methods

CHG wipes

Commercially available CHG wipes were purchased and used in this study which contains 2% (w/w) chlorhexidine gluconate (CHG). Aseptically CHG was extracted from wipes by fluid squeezing extraction method and was kept in a fridge till further processing.

CHG Bathing Solutions

Five samples of chlorhexidine solutions which ~~is~~ are used for bathing or showering pre-operative patients were collected from different clinical wards including ICU from Universiti Teknologi MARA Specialist Centre (UITMSC) and all the samples were kept aseptically in a fridge for further use.

Bacterial Strains

Five bacterial isolates were collected from UITMSC and used in this study including MRSA, *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella* sp. and *Pseudomonas aeruginosa*. Each bacterium was cultured in blood agar for 24 hours at 37°C. All tested bacteria were maintained in blood agar at 4°C and placed in the cold room.

CHG Dilution

A series of decreasing concentrations of the CHG were obtained using two-fold serial dilution method in which the original concentrations of CHG were considered 4% and 2% for bathing solution and wipes respectively. The subsequent concentration was prepared by adding 5 ml of CHG into a sterile tube with 5 ml sterile distilled water to give 50% diluted concentration. Then the rest of the concentrations were prepared in same descending manner. CHG concentrations used in this study range from 4% to 0.03% and 2% to 0.02% for bathing solution and wipes respectively.

Antimicrobial Susceptibility Testing

Agar well diffusion method was used to determine the antimicrobial activity of CHG bathing solutions and CHG wipes. One hundred microliters of bacterial suspension were spread on Muller-Hinton Agar (MHA) plates containing 6 mm wells. Fifteen microliters of 4% CHG bathing solutions and 2% CHG wipes was poured into each well and plates incubated at 37°C aerobically for 24 hours. Next day, the diameter of the growth inhibition zone around the wells was measured in millimetre and

recorded. Wells containing tested solutions with no inhibition zones were considered as negative results. Both ampicillin (10 µg) and chloramphenicol (30 µg) were used as a control.

Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC)

Broth microdilution assays were used to determine the MIC and the MBC of the CHG bathing solution and wipes against nosocomial bacterial strains as recommended by the Clinical Laboratory Standards Institute (Wayne, 2018). Different concentrations of the CHG were used. This test was performed using microplates. Different concentrations of CHG (bathing solution and wipes) and bacterial inoculums were added to each well. Plates were incubated at 37°C for 24 hours and bacterial growth was determined by microplate absorbance reader. The highest dilution of CHG that showed no visible bacterial growth was considered as MIC. The MBC of the CHG was determined by sub-culturing various concentrations of CHG and bacterial suspensions on MH agar plates. The number of surviving organisms was determined. MBC was defined as the lowest concentration that did not show any bacterial growth on those plates following the incubation period.

Results and Discussion

Surgical and intensive care units are considered a high burden on patients and hospitals. Nosocomial infection in these units is associated with high mortality, morbidity and prolonged stay in hospital, which lead to increase treatment cost (Al-Talib *et al.*, 2010; Arefian *et al.*, 2019). Possibility of getting infection is high in these units especially postoperative and medical interventions due to poor immune systems of patients admitted in ICUs or wound contamination intraoperative.

A thousand of bacteria live permanently on skin and contribute to health by maintaining a steady colony that inhibits establishment of harmful yeast and fungal infections (Webster & Osborne, 2015). In our previous study we investigated the effects of antiseptics CHX, hydrogen peroxide, iodine, ethanol and Dettol against nosocomial bacteria including MRSA, *Acinetobacter baumannii, E. coli*, *Klebsiella* species and *Pseudomonas aeruginosa* and the results showed that CHG was the second most effective antiseptics after hydrogen peroxide (Al-Talib *et al.*, 2019). In this study we want to compare and evaluate the effectiveness of CHG used in bathing solution and in antiseptics wipes against nosocomial bacteria. Many formulations were used for preoperative antiseptic preparation for patients, like using CHG bathing solution and CHG-impregnated wipes.

This study revealed that CHG bathing solution showed excellent inhibitory effects against all studied nosocomial bacteria with inhibition zones wider than respective control disc as in Table 1.

Table 1: Bacterial inhibition zones by using CHG bathing solution and CHG wipes

Bacteria	Inhibition Zone (mm)		Control Disc	Zone Size (mm)
	CHG (B)*	CHG (W)		
MRSA	25	11	Vancomycin **	12
A. Baumannii	15	0	Polymyxin B***	10
E. coli	23	15	Polymyxin B	13
Klebsiella sp.	17	10	Imipenem****	15
P. aeruginosa	20	0	Polymyxin B	13

*(B): Bathing solution, (W): Wipes
** Vancomycin 30µg, *** Polymyxin B 300U, **** Imipenem 10µg

The findings of this study supported the results of previous studies which suggested that CHG bathing solution was effective against nosocomial bacteria (Climo *et al.*, 2009; Popovich *et al.*, 2009). Using 4% CHG shower is believed to have reduced bacterial colony counts nine-fold compared with other cleaning measures (Hranjec *et al.*, 2010). The current study showed that 2% CHG wipes was effective only against *E. coli* with inhibition zone of 15 mm compared to polymyxin B with 13 mm.

Figure 1 clearly shows better performance of CHG bathing solution than wipes in terms of wider inhibitory zones for all tested bacteria. The reasons for that could be due to lower concentration of CHG wipes 2% compared to bathing solution 4% or could be due to bad storage and transport conditions of the wipes or could be due to usage of preservatives which reduced the effects of CHG in wipes.

Previous study by Graling and Vasaly (2013) on the effectiveness of preoperative bathing with CHG cloths for reducing surgical site infections indicated an overall reduction of postoperative infections for patients treated earlier with 2% CHG cloths. Another reason is it is well known that there is a poor correlation between in vitro and in vivo assays for determination of antimicrobial effects (Shi *et al.*, 2019). Thus, the urgency to determine in vitro CHG antimicrobial effects of both bathing solution and antiseptic wipes is a trend to know and compare their bacterial inhibitory effects.

Surprisingly, in an earlier study Berrondo *et al*. (2019) stated that CHG bathing/wipes add cost with no clear benefit for reducing surgical site infection among pediatric patients undergoing hernia/hydrocele repair and or orchiopexy. The earlier study is limited to certain age group and not inclusive (Berrondo *et al.*, 2019).

In this study, the MIC of CHG bathing solution was 0.03% against all study bacteria. However, the MIC of CHG against *P. aeruginosa* was 0.06% (Table 2). Similar findings were obtained in an earlier study conducted in Turkey which stated that the bactericidal activity of CHG decreased at low concentrations against

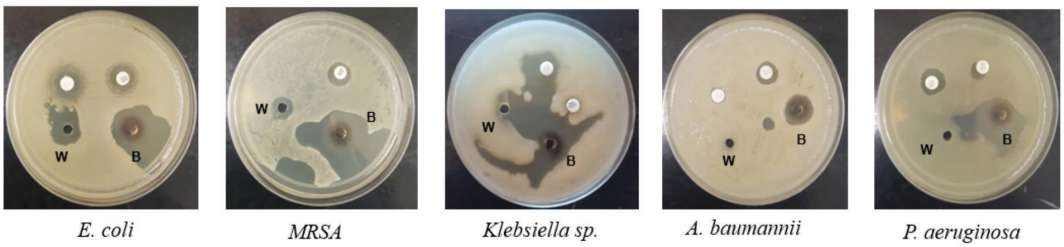

Figure 1: Antimicrobial effects of bathing solution and antiseptic wipes on nosocomial bacteria

Table 2: Comparison of MIC values for bathing solution and antiseptic wipes against nosocomial bacteria

Bacteria	MIC of CHG Bathing Solution and CHG Wipes	
	Bathing Solution	Antiseptic Wipes
MRSA	0.03	0.13
A. baumannii	0.03	NA*
E. coli	0.03	0.5
Klebsiella sp.	0.03	NA*
P. aeruginosa	0.06	NA*

*NA: No MIC against value.

P. aeruginosa (Ekizoglu *et al.*, 2016). Higher MIC of CHG antiseptic wipes was observed against MRSA and *E. coli* with 0.13% and 0.5% respectively, although no MIC was determined against *A. baumannii*, *Klebsiella sp.* and *P. aeruginosa*. This is not surprising and goes with antimicrobial susceptibility findings.

A previous study preferred to use 2% CHG antiseptic wipes over 4% CHG bathing solution based on CHG that remains on the skin after a no-rinse application of wipes (Ryder & Jodi, 2007). We believe that not only will the CHG remain on the skin after using antiseptic wipes, but there will be also more bacterial load since CHG wipes have lower antimicrobial effects.

Although CHG wipes exert an additional built-in benefit of mechanical assistance of removing bacteria and antibacterial property but earlier study by Makhni *et al.* (2018) showed no statistically significant decrease among skin bacterial population.

The MBC of chlorhexidine bathing solution against MRSA, *A. baumannii* and *E. coli* was 4%, 0.25% and 0.5% respectively Table 3. The MBC of bathing solution against *Klebsiella sp.* and *P. aeruginosa* couldn't be determined due to full growth of bacteria on MH agar. The MBC of antiseptic wipes couldn't be defined due to full growth of all study bacteria.

Conclusion

This study has confirmed that CHG bathing solution has excellent inhibitory effects against nosocomial bacteria in comparison to CHG antiseptic wipes and therefore should be recommended in preoperative procedures and medical interventions. Thorough and regular evaluation of CHG wipes should be performed before being used in hospitals.

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Table 3: Comparison of MBC values for bathing solution and antiseptic wipes against nosocomial bacteria

Bacteria	MBC of Bathing Solution	MBC of Antiseptic Wipes (%)
MRSA	4 %	++*
A. baumannii	0.25 %	++
E. coli	0.5 %	++
Klebsiella sp.	++	++
P. aeruginosa	++	++

* ++: Full growth of bacteria seen on MH agar plates

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